Mild Hypothermia Alters Midazolam Pharmacokinetics in Normal Healthy Volunteers

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ABSTRACT:

The clinical use of therapeutic hypothermia has been rapidly expanding due to evidence of neuroprotection. However, the effect of hypothermia on specific pathways of drug elimination in humans is relatively unknown. To gain insight into the potential effects of hypothermia on drug metabolism and disposition, we evaluated the pharmacokinetics of midazolam as a probe for CYP3A4/5 activity during mild hypothermia in human volunteers. A second objective of this work was to determine whether benzodiazepines and magnesium administered intravenously would facilitate the induction of hypothermia. Subjects were enrolled in a randomized crossover study, which included two mild hypothermia groups (4°C saline infusions and 4°C saline + magnesium) and two normothermia groups (37°C saline infusions and 37°C saline + magnesium). The lowest temperatures achieved in the 4°C saline + magnesium and 4°C saline infusions were 35.4 ± 0.4 and $35.8 \pm 0.3°C$, respectively. A significant decrease in the formation clearance of the major metabolite 1′-hydroxymidazolam was observed during the 4°C saline + magnesium compared with that in the 37°C saline group ($p < 0.05$). Population pharmacokinetic modeling identified a significant relationship between temperature and clearance and intercompartmental clearance for midazolam. This model predicted that midazolam clearance decreases 11.1% for each degree Celsius reduction in core temperature from 36.5°C. Midazolam with magnesium facilitated the induction of hypothermia, but shivering was minimally suppressed. These data provided proof of concept that even mild and short-duration changes in body temperature significantly affect midazolam metabolism. Future studies in patients who receive lower levels and a longer duration of hypothermia are warranted.

Mild hypothermia has been used clinically with varying success as a neuroprotective strategy in a wide array of diseases including cardiac arrest, stroke, hypoxic-ischemic encephalopathy, hepatic encephalopathy, and traumatic brain injury (Bernard et al., 2002; Hypothermia after Cardiac Arrest Study Group, 2002; Marion, 2002; Marion and Bullock, 2009). Patients are typically cooled to 32 to 34°C for a duration of 12 to 48 h in adults and up to 72 h in neonates. This degree of cooling has been shown to alter the concentration of and response to several medications, such as phenobarbital, vecuronium, phenytoin, propofol, fentanyl, and morphine (Kadar et al., 1982;

Leslie et al., 1995; Caldwell et al., 2000; Iida et al., 2001; Heier et al., 2002; Fritz et al., 2005) The mechanism of alterations in drug level and response with temperature has not been fully elucidated. Given the high rate of adverse drug events and the difficulty in identifying such events in critically ill patients (Vargas et al., 1998), determining the key factors that alter drug disposition is essential for safe and effective pharmacotherapy.

Previous experiments in animal models, as well as human studies by our laboratory and others, have implicated reduced hepatic metabolism as a mechanism of increased drug levels during cooling. Medications commonly used in critically ill patients (antiarrhythmics, β-blockers, calcium channel blockers, benzodiazepines, anesthetics, opioids, anticonvulsants, proton pump inhibitors, and others) are largely metabolized through the cytochrome P450 system and specifically by the CYP3A4/5 isoform (Tortorici et al., 2007). Studies to identify the effects of mild hypothermia on cytochrome P450 metabolism in humans are limited, and the translational significance of the observations in the rat model remains to be identified. The primary objective of our study was to evaluate the effect of temperature reduction on CYP3A4/5 activity in healthy human subjects by determining the alterations in the metabolism of midazolam.

A secondary objective of our study was to determine whether benzodiazepine administration with magnesium would facilitate the

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ABBREVIATIONS: LC, liquid chromatography; MS, mass spectrometry; CI, confidence interval; AUC, area under the curve; OFV, objective function value; TEM, core temperature.

induction of therapeutic hypothermia in conscious patients. Hypothermia is currently achieved through methods that force core temperature below the internal homeostatic set point. Forced cooling induces compensatory responses such as shivering and vasoconstriction, which are obstacles to reaching target clinical hypothermic temperatures before hospital admission (Nagao et al., 2000; Hayashi et al., 2002; Kim et al., 2009; Polderman and Herold, 2009). Magnesium sulfate has been shown to facilitate cooling and to blunt the shivering response associated with cold (4°C) saline infusion (Zweifler et al., 2004; Wadhwa et al., 2005). In the current study, we chose a factorial design to examine the effects of administration of cold (4°C) saline in conjunction with benzodiazepine sedation and magnesium sulfate-induced vasodilation as a method to induce hypothermia in conscious subjects.

Materials and Methods

Subjects. This study was approved by the University of Pittsburgh Institutional Review Board. Six healthy male subjects between the ages 19 and 39 years provided informed consent and completed all phases of the study. Each subject received a standard history, physical examination, laboratory studies (serum electrolytes, renal and liver function, thyroid-stimulating hormone, hemoglobin, and hematocrit) and a 12-lead electrocardiogram to screen for the presence of cardiac or any other underlying disease. Subjects were excluded if they had an abnormal laboratory value or any known medical problems or if they were taking any medications with the exception of seasonal allergy medication, over-the-counter nonsteroidal anti-inflammatory drugs, or acetaminophen. Other exclusion criteria included a history of cardiac disease in a family member younger than age 40, an allergy to midazolam or other benzodiazepine/narcotic medication, current smoking or recreational drug use, and a body mass index >35 kg/m². Body fat percentage was measured by three-site skinfold analysis before the first protocol visit (Pollack et al., 1980). The subjects were asked not to consume grapefruit juice and herbal dietary supplements including St. John's wort from 2 days before each study day. Alcoholic drinks and caffeine-containing food and beverages were also not allowed 24 h before or during the study.

Testing Protocol. We conducted a prospective, randomized, single-center, four-way crossover laboratory study. Testing was performed in the Emergency Responder Human Performance Laboratory under the supervision of a study physician. Mild hypothermia was induced by rapid cold (4°C) saline infusion (a total infusion of 30 ml/kg saline over 30 min) with or without 4 g of magnesium sulfate (Moore et al., 2008). The normothermia group control was given the warm saline infusion with or without magnesium. The four treatment groups were 1) 37°C saline infusion (warm), 2) 37°C saline infusion with magnesium sulfate infusion (warm + magnesium), 3) 4°C saline infusion (cold), and 4) 4°C saline infusion with magnesium sulfate infusion (cold + magnesium). When provided, magnesium was administered with the cold or warm saline infusion. The four visits were separated by at least 1 week. Subjects received a standard three-lead electrocardiogram, blood pressure monitoring, pulse oximetry, and end-tidal carbon dioxide monitoring throughout each study visit. Blood pressure and subject thermal sensation were measured at 5-min intervals throughout the infusion and every 10 min thereafter. Because our study involved minimal sedation from a dose of midazolam, a universal Ramsay sedation scale was used to evaluate the sedation state (Ramsay et al., 1974). An 18-gauge peripheral intravenous catheter was placed in the antecubital vein of each subject for the saline infusion. A second 18-gauge peripheral intravenous catheter was placed in a superficial arm or hand vein for blood sampling. After the 30-min saline infusion, a 1 mg/h lorazepam infusion was initiated in an attempt to maintain the lower core temperature for 3 h until 210 min. After the 3-h cooling maintenance period, the subject was covered with blankets and observed for an additional hour before being discharged from the laboratory. The room temperature in the laboratory was maintained at approximately 22°C throughout the study.

Temperature Measurements. Core body temperature was monitored using a precalibrated ingestible thermometer pill that continuously measures temperatures in the range of 0 to 50°C (HQ Technologies, Palmetto, FL). Temperature values recorded by this device are intermediate to esophageal and rectal temperatures (O'Brien et al., 1998). The capsule was administered to the subject 60 min before

the beginning of the protocol with approximately 30 ml of water. The protocol was initiated after three consecutive measurements indicated a stable core temperature reading. Skin surface thermistors were placed over the 1) clavicular head of pectoralis major, 2) supraspinatus, 3) triceps brachii, and 4) quadriceps femoris muscles. Core and skin surface temperatures were documented every 2 min during the infusion and every 10 min thereafter. Mean skin temperature (T_{sk}) was calculated by using the formula T_{sk} = chest (0.25) + back (0.25) + thigh (0.3) + arm (0.2) (Ayling, 1986). To mathematically describe the temperature reduction in these subjects, we estimated the area of temperature change as a reflection of cooling burden. Every cooling burden was calculated from individual time-temperature curves (trapezoid area from 0 to 210 min − the area under the temperature curve calculated from Prism).

Drug Administration and Sampling. A total of 6 mg of midazolam was given intravenously as three separate 2-mg doses. The three doses were given at 0, 10, and 20 min from the start of the saline infusion. Blood samples were collected at baseline and at 5, 15, 25, 30, 50, 80, 140, and 200 min. Specifically, the 5- and 15-min samples were before the second and third midazolam doses, respectively. The blood samples were centrifuged at 2000g for 10 min. Total urine volume was measured at the end of rewarming, and an aliquot of urine was collected from the final volume to determine 1′-hydroxymidazolam formation clearance. All plasma and urine samples were frozen at −70°C until the time of analysis.

Drug Assay. Plasma concentrations of midazolam and urine concentration of 1′-hydroxymidazolam were determined by using liquid chromatography (LC)-single quadrupole mass spectrometry (MS) (single quadrupole mass spectrometer; Thermo Fisher Scientific, Waltham, MA). A total of 150 μl of plasma was added to 0.5 M Na₂CO₃-NaHCO₃ (pH 9.6) buffer solution. Samples were then spiked with 20 μl of midazolam-d₄ (0.1 μg/ml) (Cerilliant Corporation, Round Rock, TX) as internal standard. After vortexing, 5 ml of methyl *tert*-butyl ether was added to the sample, and the sample was centrifuged at 3000g for 10 min at 4°C. The extracted supernatant was collected in a glass tube and evaporated to dryness under a gentle stream of nitrogen, followed by reconstitution in 200 μl of the mobile phase, which consisted of 0.1% formic acid in water and acetonitrile. A volume of 20 μl of each sample was injected onto an RP18 5 μm, 2.1 × 150 mm high-performance liquid chromatography column (Thermo Fisher Scientific). The mass spectrometer was operated in the positive ion mode for detection of the protonated molecular ions [M + 1]⁺ with a cone temperature of 350°C and a capillary voltage of 2200 V. Positive ions at *m/z* for midazolam, 1′-hydroxymidazolam, and midazolam-d₄ were 325.9, 341.9, and 330.7, respectively. To measure the 1′-hydroxymidazolam concentration in the urine, urine samples were incubated with 2000 IU of glucuronidase (Sigma-Aldrich, St. Louis, MO) with 0.2 M acetate buffer (pH 4.9) at 37°C for 24 h. The extraction and LC/MS analytical procedure were similar to the midazolam method described above. Calibration curves ($r^2 \geq$ 0.99) for midazolam (3–300 ng/ml) and 1′-hydroxymidazolam (100–2000 ng/ml) were linear. Midazolam and 1′-hydroxymidazolam analytical LC/MS methods have been validated with <9.5 and <8.3% interday precision and <7.2 and <7.3% intraday precision over all concentrations, respectively. Recoveries of midazolam and 1′-hydroxymidazolam from liquid-liquid extraction were >89.2 and >91.7% over all concentrations, respectively.

Physiologic Data Analyses. Repeated temperature measurements (core and skin temperatures), heart rate, respiratory rate, and end-tidal CO₂ were compared by generalized estimating equations using the factors of time, cold (4°C) saline infusion, magnesium infusion, and the interaction of cold (4°C) and magnesium. The estimated effect for each factor is presented as the coefficient estimate (*B*) with 95% confidence interval (95% CI). Analyses were performed with Prism (release 4.0c).

Noncompartmental Pharmacokinetic Analysis. Noncompartmental analysis (WinNonlin Professional, version 4.01; Pharsight, Mountain View, CA) was used to fit the time-concentration data and obtain the estimation for the area under the curve from time 0 to infinity (AUC₀₋ᵢₙf), area under the curve from time 0 to the last sample time (AUC₀₋T), systemic clearance (CLₛ), estimated half-life ($t_{1/2}$), elimination rate (K_e), and volume of distribution (V_z). Formation clearance (CL_f) of 1′-hydroxymidazolam was calculated by dividing the amount in the urine (1′-hydroxymidazolam concentration in the urine × total urine volume) by the plasma midazolam AUC₀₋T. One-way repeated analysis of variance with Bonferroni post hoc analysis was used to compare formation clearance in the four treatments. Significance was denoted by $p < 0.05$.

Population Pharmacokinetic Modeling. The population pharmacokinetic model describing midazolam disposition was constructed using a nonlinear

mixed-effects approach as implemented in the NONMEM V1.1 program (Icon, Hanover, MD). The population pharmacokinetics model consists of a pharmacokinetics structural model and a statistical model in which between-subject and within-subject variabilities are described. One- and two-compartment structural pharmacokinetics models were investigated as base model structures. The one-compartment model structure was evaluated using the ADVAN1 TRANS2 routine, and the two-compartment model structure was implemented as the ADVAN3 TRANS4 subroutine. The first-order conditional estimation method with interaction was used to estimate all parameters. The interindividual variability in the pharmacokinetic parameters was assumed to be log normally distributed. The residual variability was evaluated using three candidate model structures: additive, proportional, and a combined additive and proportional model $Y_{ij} = F_{ij} \times [1 + Err(1)] + Err(2)$.

The model building process was guided by analyzing the goodness-of-fit plots, precision of parameter estimates, and the objective function value (OFV) provided by NONMEM. After the base model was selected, we evaluated the effect of core temperature (TEM), heart rate, and magnesium effect (yes or no) on the pharmacokinetic parameters (CL, V, or CL, V_1, V_2, Q). Covariate effects were evaluated using a forward stepwise addition and reverse deletion approach. The impact of the covariates were evaluated using the change in the $-2 \times$ log likelihood, visual diagnostics, successful minimization, parsimony, and physiologic reasonableness of the covariate effects. Improvements in the model were accepted as significant

($p < 0.01$, 1 df) when a decrease of >6.64 points in the OFV per added model parameter was observed. Diagnostic plots included population-predicted concentrations versus observed concentration, individual predicted concentrations versus observed concentration, population-predicted concentrations versus weighted residuals and time versus weighted residuals. Empirical Bayes estimates for individual patient pharmacokinetic parameter clearances were generated and compared with the results from the noncompartmental analysis.

Bootstrap Evaluation. A nonparametric bootstrap approach using sampling with replacement was used to assess the robustness of the model estimates (Parke et al., 1999). Sampling with replacement involved creating a series of datasets of size equal to the original data set generated by repeatedly sampling individuals from the original data set, removing these individuals, and replacing them at random. The model was refitted to each new data set, and this process was repeated 1000 times. The stability of the final model was evaluated by examining the 95% CI of model parameter estimates. The Wings for NONMEM (G77 Fortran with WFN 408b) implementation for bootstrapping was used (Hayes et al.,1989).

Simulation. The population parameter estimates obtained from the final model were used to simulate the population average concentration-time profiles at three fixed core temperatures (36.5, 34, and 32°C) over a duration of 400 min. A 2-mg dose of midazolam was administered in the simulation as three intravenous doses (as used in this study). The simulations were implemented in WinNonlin 4.01 with use of the population-estimated parameters from NONMEM.
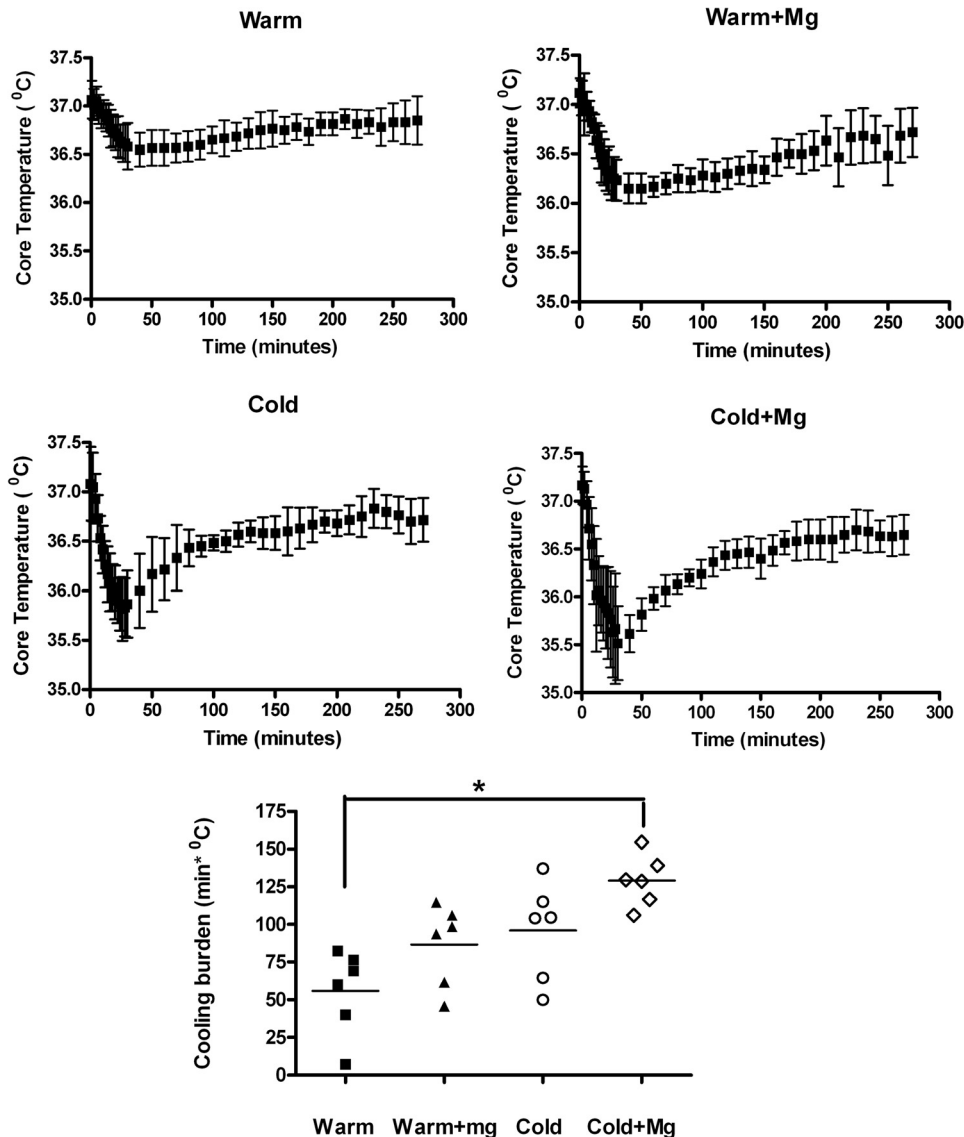

FIG. 1. Core temperature (mean ± S.D.) over time curves of four treatment groups. The temperatures were recorded every 2 min during the first 30 min and every 10 min thereafter. The cold (4°C) burden area was calculated from each individual temperature-time curve. There was a significant difference between warm and cold + magnesium groups ($p = 0.01$) of the cold burden area. ■, warm; ▲, warm + magnesium; ○, cold; ◇, cold + magnesium.

Results

Subjects and Physiologic Variables. Eight subjects provided informed consent and were screened for this study. Two subjects were excluded during screening because of laboratory values that were outside the normal range. The demographics and morpho-metrics of six subjects were as follows: age, 28.5 ± 7.6 years; height, 175.7 ± 4.7 cm; mass, 78.8 ± 8.6 kg; and body fat, 17.3 ± 3.2%. The procedure was well tolerated in all subjects. In our study, we observed relatively minimal sedation with ventilatory and cardiovascular functions unaffected by midazolam. No subject
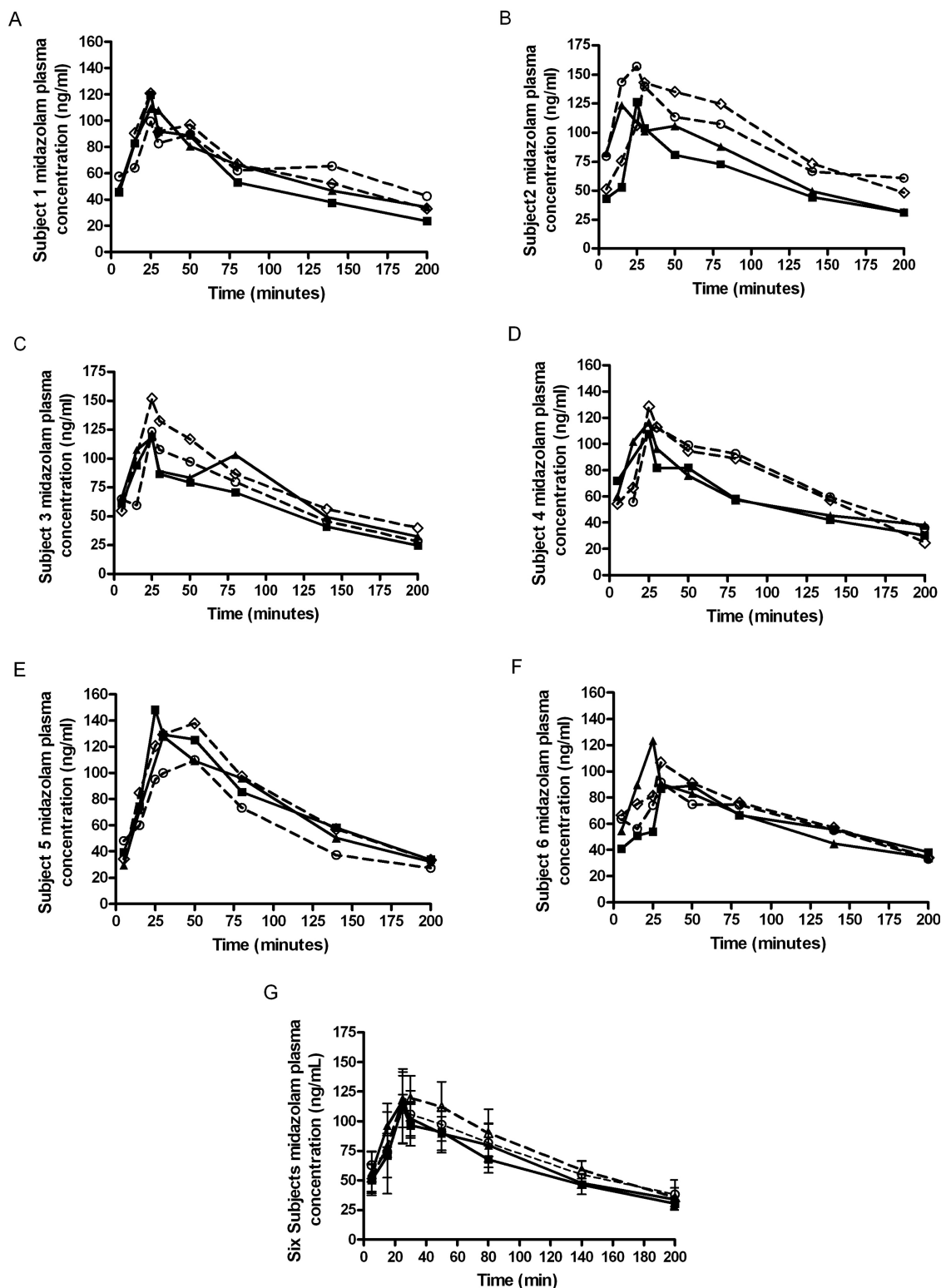

FIG. 2. Individual subject midazolam time-plasma concentration profile from the four treatments (A–F) and the average time-concentration curves for six subjects (mean ± S.D.) (G). Cold (4°C) infusions are denoted by dashed lines, and warm infusions are denoted by solid lines. ■, warm; ▲, warm + magnesium; ○, cold; ◇, cold + magnesium.

exceeded a value of 3 (patient awake, responds to commands only) on the Ramsay sedation scale. There was an effect of magnesium [$B = 8.18$ (1.66–14.7), $p = 0.014$] on heart rate. There was no effect of cold (4°C) saline, magnesium, or time on respiratory rate and end-tidal CO_2.

Temperature. Rapid infusion of 37°C saline resulted in a 0.4 ± 0.2°C decrease from baseline temperature. Infusion of 37°C saline with magnesium resulted in a temperature reduction of 0.9 ± 0.3°C. Infusion of cold (4°C) saline reduced core temperature 1.4 ± 0.3°C from baseline with a mean nadir temperature of 35.8 ± 0.3°C. Addition of magnesium to the cold (4°C) infusion reduced core temperature 1.8 ± 0.3°C from baseline with the mean nadir at 35.4 ± 0.4°C. In cold and cold + magnesium groups, the duration (time below the 36.3°C, which is the lowest value in the warm saline infusion groups) of the mild hypothermia group was 47.0 ± 24.5 and 101.3 ± 26.6 min, respectively. Statistically, there was an effect of cold saline [$B = -0.48$ (−0.87 to −0.92), $p = 0.015$] and magnesium [$B = -0.40$ (−0.77 to −0.02), $p = 0.035$] on core temperature. There was neither a time nor a cold saline/magnesium interaction on core temperature. Mean skin temperature changed over time [$B = -0.007$ (−0.01 to 0.0), $p = 0.004$] but did not differ from the temperature of the infusion or with addition of magnesium. Cooling burden was calculated, and a significant difference was found between warm and cold + magnesium groups ($p = 0.01$). The core temperature curve and the cooling burden are shown in Fig. 1.

Midazolam Time-Concentration Profile and Noncompartmental Analysis. Six individual midazolam time-plasma concentration profiles for all four treatments are shown in Fig. 2. The estimated C_{max}, AUC_{0-inf}, CL_s, CL_f, $t_{1/2}$, K_e, and V_z of the four treatments from noncompartmental analysis are shown in Table 1. A significant decrease in the 1′-hydroxymidazolam formation clearance was observed during cold + magnesium compared with the warm group (2.43 ± 0.782 versus 3.41 ± 0.735 ml/min/kg, $p = 0.0168$). Systemic clearance during cold + magnesium compared with the warm saline group demonstrated a trend toward a significant reduction during hypothermia versus normothermia (3.76 ± 0.386 versus 4.49 ± 0.560 ml/min/kg, $p = 0.0568$). No significant difference in C_{max}, $t_{1/2}$, AUC_{0-inf}, and V_z was seen among the four treatments. Individual 1′-hydroxymidazolam formation clearance and midazolam systemic clearance of four treatments are depicted in Fig. 3.

Population-Based Nonlinear-Mixed Effect Pharmacokinetic Modeling. The data set comprised 258 plasma concentrations from 24 visits. A two-compartment base model structure was significantly better at describing the disposition of midazolam than the one-compartment model (OFV difference 16.0 points, 2 df, $p < 0.001$). The final two-compartment model including covariate relationships is as follows: CL_s (liters per hour) = $18.5 \times (TEM/36.5)^{4.24}$, V_1 (liters) = 9.11, Q (liters per hour) = $230 \times (TEM/36.5)^{-17.9}$, V_2 (liters) = 37.3 + (heart rate/68)$^{-5.08}$ + magnesium × (−3.75).

The final model had an OFV of 1152.0, 28.9 units lower than the best base model OFV of 1180.9. Graphic model performance is depicted in Fig. 4. Both systemic clearance and intercompartment clearance of midazolam were affected by core temperature. Temperature was not a significant covariate when tested in relation to the V_1 and V_2 parameters. Heart rate and magnesium significantly affected V_2. The presence of magnesium reduced the volume of distribution by 3.75 liters (close to 10% volume of V_2). V_2 values changed 73.13 and 75.06% from the maximum to the minimum observed heart rate with or without magnesium, respectively. The pharmacokinetic parameters estimated from the NONMEM analysis were consistent with those from the noncompartmental analysis. Based on this model, the lowest core temperature (34.8°C) in this study would result in a CL_s of 16.0 l/h, which was 29.6% lower than the clearance of 22.7 l/h from the highest core temperature (37.8°C). As temperature decreases, midazolam elimination from the central compartment declines. The model describes an estimated 11.1% reduction in midazolam clearance for every 1°C reduction in core temperature from 36.5°C. The individual level model-predicted relationships (based on the empirical Bayes estimates) between specific temperature and midazolam clearance are shown in Fig. 5.

Bootstrapping and Simulation. The final model was assessed further by nonparametric bootstrapping method, and the results of bootstrap estimates were consistent with NONMEM estimates, which means the final model was relatively stable and robust. NONMEM estimates and the 95% CI from bootstrapping are shown in Table 2. A concentration-time profile of midazolam simulated from the population level NONMEM parameters is shown in Fig. 6. The simulation curve for a core temperature 32°C has the highest AUC and C_{max} followed by 34 and 36.5°C. The simulations reflect the model-predicted reductions in midazolam clearance at 32°C (a 42.8% reduction compared with that at 36.5°C) and 34°C (a 26.0% reduction compared with that at 36.5°C).

Discussion

This study demonstrated that midazolam metabolism is reduced by mild short duration of hypothermia in normal healthy volunteers. These results provided the basis for estimating changes in midazolam clearance under mild hypothermic conditions. There are two major observations from this study. First, the cold (4°C) saline 30 ml/kg infusion with midazolam and magnesium reduced the core temperature in normal healthy volunteers in the absence of anesthesia; however, shivering was minimally suppressed in healthy subjects. Second, the clearance of midazolam was significantly reduced by decreasing body temperature in humans as determined by both noncompartmental pharmacokinetic analysis and population pharmacokinetic modeling. This model predicted that midazolam clearance would decrease 11.1% for each degree lower in core temperature from 36.5°C.

TABLE 1

Pharmacokinetic variables for four treatments from noncompartmental analysis

One-way repeated analysis of variance was used to detect the differences among the four treatment groups. Data presented as mean (S.D.).

	Warm	Warm/Magnesium	Cold	Cold/Magnesium	p
C_{max} (ng/ml)	123 (23.1)	120 (6.88)	116 (23.0)	132 (16.2)	0.360
$t_{1/2}$ (min)	107 (20.8)	107 (20.2)	119 (29.4)	96.3 (9.50)	0.212
AUC_{0-T} (ng · min/ml)	13.8 (1.64)	15.1 (1.16)	16.0 (2.90)	17.1 (2.27)	0.0364*
AUC_{0-inf} (ng · min/ml)	17.1 (1.63)	18.7 (0.788)	20.8 (4.33)	20.6 (2.33)	0.0649
V_z (ml)	54.5 (11.7)	49.8 (9.59)	50.3 (10.6)	41.4 (7.11)	0.0908
CL_s (ml/min/kg)	4.49 (0.560)	4.12 (0.492)	3.83 (0.881)	3.76 (0.386)	0.0568
CL_f (ml/min/kg)	3.41 (0.735)	2.65 (0.923)	2.56 (0.917)	2.43 (0.782)	0.0168*

* $p < 0.05$.

The body temperature changes observed in this study from both normothermia and hypothermia groups were consistent with those in previous studies. In our normothermia group, warm saline infusion with midazolam resulted in a small 0.4 ± 0.2°C decrease from baseline. Addition of magnesium resulted in a temperature reduction of 0.9 ± 0.3°C. Kurz et al. (1995) used midazolam and surface cooling to induce hypothermia and showed that midazolam impairs

FIG. 3. 1′-Hydroxymidazolam formation clearance and midazolam systemic clearance from noncompartmental analysis estimation with mean for four treatment groups. A difference was observed in 1′-hydroxymidazolam formation clearance between warm and cold + magnesium ($p = 0.0168$). A trend toward significance between warm and cold + Mg for systemic clearance ($p = 0.0568$). ■, warm; ▲, warm + magnesium; ○, cold; ◇, cold + magnesium.

thermoregulatory control by decreasing the sweating threshold 0.3°C and decreasing the shivering threshold by approximately 0.6°C. Our previous study using a rapid warm saline infusion alone resulted in a 0.5°C temperature reduction (Moore et al., 2008). In our hypothermia group, the lowest core temperature achieved was 35.4°C ± 0.4°C, in the cold saline infusion with magnesium group; coinfusion of 4 g of magnesium during a cold saline infusion resulted in an additional 0.5°C of cooling compared with cold saline infusion itself. We reported previously that rapid infusion of cold saline can effectively reduce body temperature in normal healthy volunteers compared with surface cooling (Moore et al., 2008; Hostler et al., 2009). Magnesium sulfate facilitates cooling through its known vasodilatory effects to promote peripheral heat exchange and blunt the shiver response upon cold saline infusion. Magnesium sulfate administered as a 8.75 to 16.75 g i.v. infusion has been shown to increase the rate of hypothermia and improve the comfort of healthy subjects during hypothermia induction (Zweifler et al., 2004). Previous studies have shown that magnesium sulfate significantly reduced the shivering threshold (36.3 ± 0.4 versus 36.6 ± 0.3°C, $p = 0.04$) in healthy volunteers (Wadhwa et al., 2005), which was consistent with the observation in this study. Therefore, magnesium use in hypothermia deserves further study. Although the gain in temperature changes was small in this study, the infusion was well tolerated in this population and may prove to be beneficial in achieving a target temperature in conscious patients. These results suggested that it is possible to reduce body temperature in conscious subjects; however, the cold saline infusion with midazolam and magnesium combination used in this study only produced a transient reduction in body temperature and did not sufficiently maintain hypothermia to a clinically desired level.

In this study, we evaluated the model compound midazolam as an index of CYP3A4/5 metabolism. Midazolam is a well known CYP3A probe, mostly because it is metabolized by human CYP3A4/5 isoforms, the metabolism is not flow dependent (low to medium extraction ratio), and midazolam is not a P-glycoprotein transporter sub-
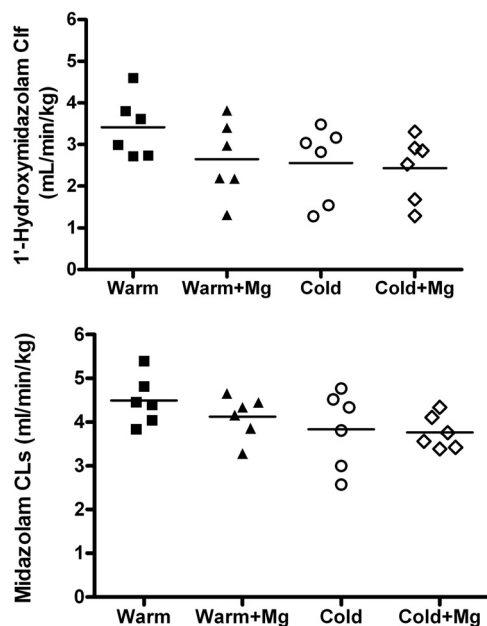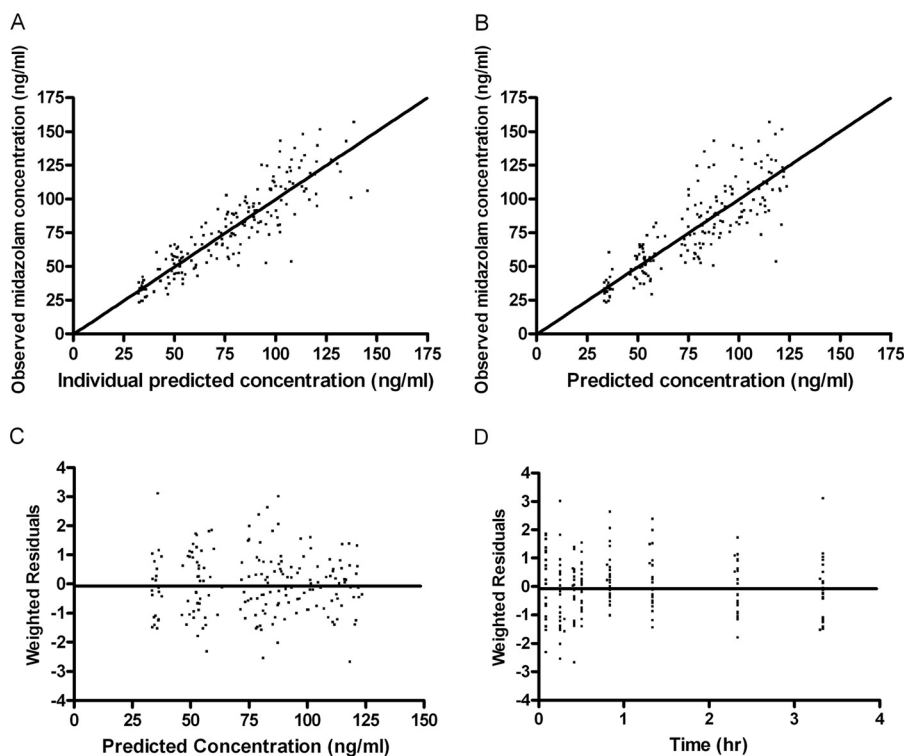

FIG. 4. Model diagnostic plots. Goodness-of-fit plots for the population pharmacokinetic model from NONMEM analysis. Individual predicted concentrations versus observed concentrations and the predicted concentrations versus observed concentrations are shown. A and B, the straight lines are the lines of unity. C and D, population-predicted concentrations versus weighted residual and time versus weighted residual.

strate. The primary midazolam metabolite produced through CYP3A in humans is 1′-hydroxymidazolam (∼70%). Minor metabolites formed by CYP3A4 are 4′-hydroxymidazolam and 1′,4′-dihydroxymidazolam (Galetin et al., 2005). Our results demonstrated that even in mild hyperthermia of short duration, the 1′-hydroxymidazolam formation clearance in the cold + magnesium group was significantly lower compared with that for normothermia. These results were consistent with previous preclinical and clinical studies. CYP3A activity during cooling was determined previously by ethylmorphine N-demethylation in vitro in piglet liver microsomes. This study demonstrated a strong temperature dependence with CYP3A activity ($p <$ 0.01) (Fritz et al., 2005). Furthermore, this study indicated that the plasma concentration of fentanyl, another CYP3A substrate (liver blood flow-dependent), significantly increased during hypothermia (31.6 ± 0.2°C) for 6 h and after rewarming ($p < 0.001$ and $p < 0.05$, respectively). Fukuoka et al. (2004) reported significant changes in midazolam clearance during moderate (32–34°C) hypothermia in eight brain-injured patients who were given a continuous infusion of midazolam. Collectively, previous preclinical or clinical studies have indicated possible changes in CYP3A activity in cooling; however, the relationship of body temperature and CYP3A activity in humans was still not clearly elucidated.

Our data analysis comprehensively estimated the pharmacokinetic parameters of midazolam during mild hypothermia in humans and predicted the relationship between core temperature and CYP3A activity by using both noncompartmental pharmacokinetic analysis and population-based nonlinear mixed-effect pharmacokinetic mod-

eling. The noncompartmental pharmacokinetic analysis assumed that the clearance was constant over time within-group, which may not be a sufficient and sensitive estimate for the changes in systemic clearance with changing temperature. Our study was designed to record core temperature and midazolam concentration continuously over time, therefore, allowing the time-varying covariate of temperature to be modeled using a nonlinear mixed-effects population pharmacokinetic approach. This approach provides for the preservation of data structure so that each individual condition contributes to the population description. The model estimate of clearance of midazolam was 18.5 l/h at 36.5°C, which was consistent with previous studies (Bolon et al., 2003; de Wildt et al., 2003; Shimizu et al., 2007). The relationship between core body temperature and the midazolam systemic clearance was described using the function, CL (liters per hour) = $18.5 \times (TEM/36.5)^{4.24}$, which provided the basis for predicting the temperature relationship with clearance of midazolam. This population model predicts that a 1°C reduction in core temperature from 36.5°C produces an 11.1% reduction in midazolam clearance. This 11.1% reduction per 1°C change in core temperature is consistent with the results of Leon (2004), who reported that for each 1°C decrease in temperature, a 10% reduction in tissue metabolic requirements and free radical production was seen. In addition, Caldwell et al. (2000) reported that 11.3% clearance of vecuronium decreased per degree (Celsius) temperature change in healthy human volunteers. Michelsen et al. (2001) also demonstrated that the clearance of remifentanil decreased by 6.37% for each degree (Celsius) below 37°C in patients who are undergoing coronary artery bypass surgery. The model suggested a predictive decrease in drug metabolism per degree change in body temperature. The predictive utility of these estimates in a prospective validation cohort remains to be determined.

One limitation of this study was the short duration of plasma samples collection for determination of midazolam PK parameters. Due to the shivering response and discomfort of healthy human subjects, the cooling period of this study was designed for a duration of 200 min. There was a likelihood of alterations in clearance upon rewarming; thus, blood samples were only drawn during the period of time when subjects were actively on the cooling protocol. This duration may not be long enough to fully capture the midazolam disposition curve; however, sampling at later time points would be confounded by greater increases in body temperatures. Despite this limitation, it is important to note that the PK parameter estimates for half-life and systemic clearance in the healthy normothermia control
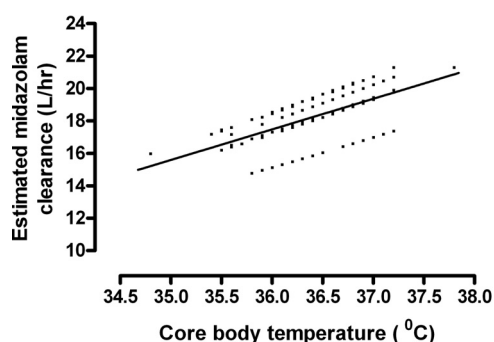
FIG. 5. The relationship between core body temperature (from the highest body temperature 37.8°C to the lowest body temperature 34.8°C observed in this study) and individual midazolam systemic clearance estimated from the final population pharmacokinetic model.

TABLE 2

NONMEM and bootstrap estimates

Data for bootstrap are mean (95% CI).

	NONMEM Estimates[a]	Bootstrap
THETA(1)	18.5	18.4 (18.36 to 18.44)
THETA(2)	9.11	9.68 (9.39 to 9.98)
THETA(3)	230	253 (202 to 304)
THETA(4)	37.3	36.7 (36.5 to 37.0)
THETA(5)	4.24	4.11 (3.86 to 4.37)
THETA(6)	−17.9	−22.1 (−23.3 to 20.8)
THETA(7)	−5.08	−4.68 (−4.81 to 4.55)
THETA(8)	−3.75	−4.12 (−4.21 to 4.03)
ETA(1)	0.0762	0.0618 (0.059 to 0.064)
ETA(2)	0.0543	0.487 (0.466 to 0.509)
ETA(3)	0.135	0.100 (0.097 to 0.103)
ERR(1)	0.166	0.169 (0.168 to 0.171)
ERR(2)	0.0992	1.64 (1.53 to 1.75)

[a] NONMEM modeling control stream: TVCL = THETA(1) · $(TEM/36.5)^{THETA(5)}$; CL = TVCL · EXP(ETA(1)); TVV1 = THETA(2); V1 = TVV1 · EXP(ETA(2)); TVQ = THETA(3) · $(TEM/36.5)^{THETA(6)}$; Q = TVQ; TVV2 = THETA(4)+$(HR/68)^{THETA(7)}$+MG · THETA(8); V2 = TVV2 · EXP(ETA(3)); S1 = V1, Y = F + F · ERR(1) + ERR(2).
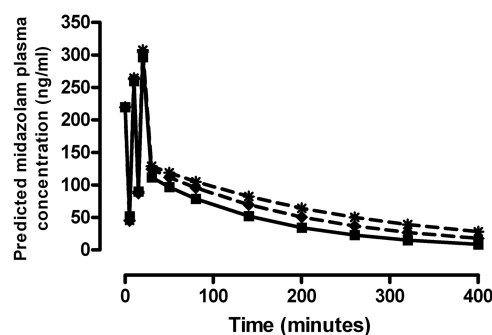
FIG. 6. The simulated time-concentration profiles of midazolam in core temperature 32, 34, and 36.5°C. The curves were generated from WinNonlin software based on the population pharmacokinetic parameters estimated from NONMEM. The simulation curve for a core temperature 32°C (∗) has the highest AUC and C_{max} followed by 34°C (♦) and 36.5°C (■). The simulations reflect the model-predicted reductions in midazolam clearance at 32°C (a 42.8% reduction compared with that at 36.5°C) and 34°C (a 26.0% reduction compared with that at 36.5°C).

group of this study was consistent with literature values as mentioned previously.

Future studies to determine specific dosing guidelines for commonly used medications in critically ill patients during cooling are needed to prevent the potential therapy-drug interactions in this highly susceptible patient population. The magnitude of pathway-specific alterations in drug elimination are necessary for subsequent studies to determine specific dosing recommendations. Until such guidelines are developed, vigilance with drug response monitoring is a clinical necessity in critically ill patients receiving therapeutic hypothermia.

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